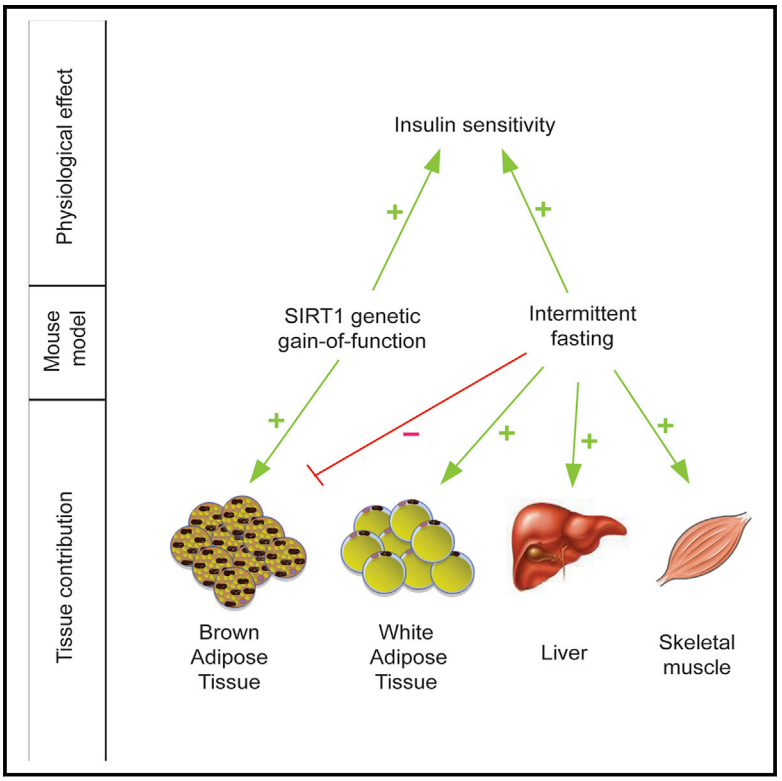
Cell Reports

SIRT1 Gain of Function Does Not Mimic or Enhance the Adaptations to Intermittent Fasting

Graphical Abstract



Authors

Marie Boutant, Sameer S. Kulkarni, Magali Joffraud, Frédéric Raymond, Sylviane Métairon, Patrick Descombes, Carles Cantó

Correspondence

carlos.cantoalvarez@rd.nestle.com

In Brief

SIRT1 has been proposed to mediate the metabolic benefits of calorie restriction (CR), suggesting that SIRT1 activation could be a CR-mimetic strategy. Here, Boutant et al. demonstrate that SIRT1 activation neither mimics nor boosts the transcriptional or metabolic adaptations to intermittent fasting, a form of CR.

Highlights

- Moderate SIRT1 overexpression does not enhance the metabolic effects of EODF
- Transcriptional responses to SIRT1 activation and EODF differ in multiple tissues
- Mitochondrial function is differentially affected by SIRT1 activation and EODF
- SIRT1 and EODF lead to opposite effects on brown adipose tissue function

Accession Numbers

GSE70857

Boutant et al., 2016, Cell Reports *14*, 2068–2075
March 8, 2016
http://dx.doi.org/10.1016/j.celrep.2016.02.007

OPEN ACCESS
CellPress

Cell Reports
Report

SIRT1 Gain of Function Does Not Mimic or Enhance the Adaptations to Intermittent Fasting

Marie Boutant,[1] Sameer S. Kulkarni,[1] Magali Joffraud,[1] Frédéric Raymond,[1] Sylviane Métairon,[1] Patrick Descombes,[1,2] and Carles Cantó[1,2,*]
[1]Nestlé Institute of Health Sciences (NIHS), 1015 Lausanne, Switzerland
[2]École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland
*Correspondence: carlos.cantoalvarez@rd.nestle.com
http://dx.doi.org/10.1016/j.celrep.2016.02.007

SUMMARY

Caloric restriction (CR) has been shown to prevent the onset of insulin resistance and to delay age-related physiological decline in mammalian organisms. SIRT1, a NAD$^+$-dependent deacetylase enzyme, has been suggested to mediate the adaptive responses to CR, leading to the speculation that SIRT1 activation could be therapeutically used as a CR-mimetic strategy. Here, we used a mouse model of moderate SIRT1 overexpression to test whether SIRT1 gain of function could mimic or boost the metabolic benefits induced by every-other-day feeding (EODF). Our results indicate that SIRT1 transgenesis does not affect the ability of EODF to decrease adiposity and improve insulin sensitivity. Transcriptomic analyses revealed that SIRT1 transgenesis and EODF promote very distinct adaptations in individual tissues, some of which can be even be metabolically opposite, as in brown adipose tissue. Therefore, whereas SIRT1 overexpression and CR both improve glucose metabolism and insulin sensitivity, the etiologies of these benefits are largely different.

INTRODUCTION

The increasing prevalence of obesity, cardiovascular disease, and type 2 diabetes mellitus in modern societies is heavily influenced by environmental and behavioral cues. Increased caloric intake and decreased physical activity are associated with a higher susceptibility to develop metabolic disorders. In contrast, calorie restriction (CR), either as reductions in daily caloric intake to ~40% below ad libitum (AL) levels or as every-other-day feeding (EODF), can delay the onset of metabolic and age-related diseases in a wide variety of organisms, including primates (Mercken et al., 2012). On practice, however, adherence to a CR regime is difficult in humans. Therefore, there is a high interest in understanding the mechanisms underlying CR in order to develop strategies that mimic or amplify its benefits.

SIRT1 is a NAD$^+$-dependent protein deacetylase and the best studied mammalian homolog of Sir2, a protein that influences longevity in yeast. In mammals, a 40% reduction in daily caloric intake leads to SIRT1 activation in diverse cells and tissues, including muscle, brain, and adipocytes (Chen et al., 2008; Cohen et al., 2004). SIRT1 deficiency leads to dwarfism, metabolic inefficiency, and failure to metabolically and behaviorally adapt to multiple CR regimes (Boily et al., 2008; Chen et al., 2005; McBurney et al., 2003; Mercken et al., 2014). In addition, CR fails to enhance rodent lifespan in SIRT1 knockout (KO) mice (Mercken et al., 2014). This evidence suggests that SIRT1 is required for multiple adaptations to CR in mice. However, whether SIRT1 activation is enough to mimic CR remains unclear. While the specificity of small-molecule SIRT1 activators (STACs), including resveratrol, has been called into question (Pacholec et al., 2010), their administration to aged mice leads to overlapping effects with CR, as it reduces age-related physical decline and mimics gene expression patterns induced by both EODF and canonical CR (Pearson et al., 2008). Here, we explored whether SIRT1 moderate overexpression, as a refined SIRT1 gain-of-function model, could mimic or boost the metabolic benefits of EODF in mice. We demonstrate that EODF adaptations are not enhanced by a moderate SIRT1 overexpression. Furthermore, our results indicate that, while both SIRT1 overexpression and EODF led to improvements on glucose metabolism, the origin of these benefits is different.

RESULTS

CR, SIRT1 Transgenesis, and Insulin Sensitivity in Mice

Wild-type (WT) and SIRT1$^{Tg/Tg}$ (Tg) mice were fed AL or subjected to EODF during 12 weeks. Body weight (BW) evolution was followed weekly during the experiment. EODF mice ate ~40% more during their meal day, leading to an overall ~30% decrease in caloric intake over 48 hr, with no differences between genotypes (Figure 1A). Whereas the BW of mice fed AL increased during the 12-week period, the mice under EODF maintained their BW around their initial values (Figure 1B). The changes in BW and composition induced by EODF were similar

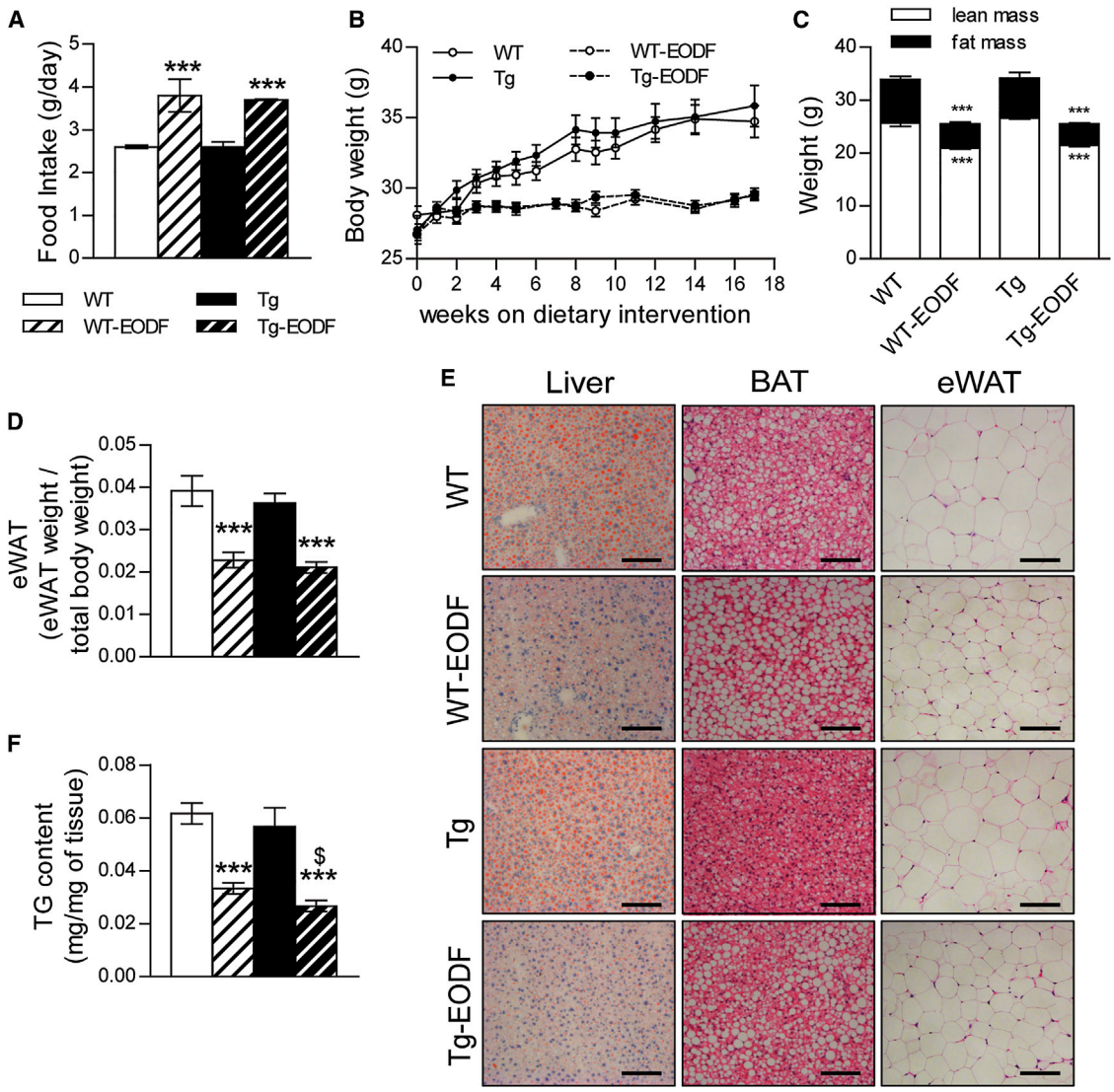


Figure 1. SIRT1 Overexpression Does Not Mimic the Beneficial Effects of EODF

12-week-old WT and Tg mice were fed AL or submitted to EODF during 12 weeks (named as WT, WT-EODF, Tg, or Tg-EODF).

(A and B) Food intake (A) and body weight evolution (B) during the experiment.

(C) Body composition was evaluated by EchoMRI 14 weeks after the initiation of EODF.

(D) eWAT weight, measured upon sacrifice.

(E) H&E staining on liver, BAT, and eWAT. Scale bars, 100 μm.

(F) Liver tissue was extracted upon sacrifice, and triglyceride levels were evaluated as described in the Supplemental Experimental Procedures.

All values are presented as mean ± SEM of n = 6–12 mice for each genotype and group. $^{\$}p < 0.05$, significant difference between genotypes. $^{*}p < 0.05$, significant difference between AL- and EODF-fed mice; $^{***}p < 0.01$, significant difference between AL- and EODF-fed mice.

in Tg and WT mice (Figures 1B and 1C). The lower fat mass content in EODF mice was paralleled by a reduction of the epididymal white adipose tissue (eWAT) weight (Figures 1C and 1D), associated with an evident decrease in the adipocyte size (Figure 1E). EODF also lowered hepatic lipid accumulation, as indicated by decreased triglyceride (TG) content and lipid droplet size (Figures 1E and 1F). While no differences in TG content were observed between WT and Tg mice fed AL (Figure 1F), Tg mice displayed a slightly more pronounced decrease of TG content in the liver upon EODF (Figure 1F). As previously described (Boutant et al., 2015), the brown adipose tissue (BAT) of Tg mice was characterized by reduced lipid droplet size (Figure 1E). Surprisingly, EODF markedly increased lipid droplet size in both WT and Tg mice (Figure 1E), rendering BAT histological appearance similar between genotypes.

Diverse CR regimes have been shown to increase the activity and decrease the heat production of mice (Bartfai and Conti, 2012; Chen et al., 2005), which was confirmed in our WT and Tg mice under the EODF paradigm (Figures 2A and 2B). This decreased heat production is in line with the increased lipid

droplet size in the BAT of EODF mice (Figures 1E and 2B). Conversely, a clear tendency ($p = 0.06$) toward increased heat production in AL-fed Tg mice was observed during the light phase (Figure 2B), in line with the lower size of their BAT lipid droplets (Figure 1E) and with the higher daily energy expenditure of these animals (Boutant et al., 2015). CR also has a strong impact on glucose homeostasis (Mercken et al., 2012). In agreement, glucose tolerance and insulin sensitivity were highly improved in EODF mice (Figures 2C and 2D). While Tg mice displayed enhanced glucose tolerance and insulin sensitivity when fed AL, the glucose excursion curves after a glucose or an insulin tolerance test were similar between WT and Tg mice upon EODF (Figures 2C and 2D; Figure S1). These data suggest that EODF prevents BW gain and improves insulin sensitivity similarly in WT and Tg mice.

Tg mice display enhanced glucose tolerance due to a higher insulin response in BAT (Boutant et al., 2015). In order to evaluate whether EODF prompted similar adaptations, we performed hyperinsulinemic-euglycemic clamps comparing AL and EODF mice. As expected, EODF improved insulin sensitivity and led to dramatic increases in glucose infusion rates in order to maintain euglycemia during the clamp (Figure 2E). This was due to a larger ability of insulin to inhibit hepatic glucose output in EODF mice, as well as to higher glucose disposal by peripheral tissues (Figures 2F and 2G), most particularly, WAT and skeletal muscle (Figure 2G). Surprisingly, EODF led to an ~45% decrease in insulin-stimulated glucose uptake in BAT (Figure 2G). This is in opposition to the observations in Tg mice (Boutant et al., 2015) and fits the contradictory histologic appearance of the BAT from EODF and Tg mice (Figure 1E). These observations provide evidence that EODF and SIRT1 trigger benefits on glucose homeostasis via different mechanisms.

The Effects of CR and SIRT1 Transgenesis on Mitochondrial Function

Next, we evaluated whether EODF altered SIRT1 expression. In contrast to 40% CR protocols (Chen et al., 2008; Cohen et al., 2004), EODF did not modulate SIRT1 protein content (Figure 3A; Figure S2A). In all tissues tested, SIRT1 was predominantly found as a cytosolic protein, to the point that SIRT1 was almost undetectable in the nuclear fraction of BAT and muscle (Figures S2B and S2C; data not shown). EODF did not alter SIRT1 distribution, at least in liver tissue (Figure S2D). Similarly, SIRT1 was almost exclusively localized in the cytosol of WT or Tg primary brown adipocytes, and its levels and localization were not affected by glucose availability (Figure S2E).

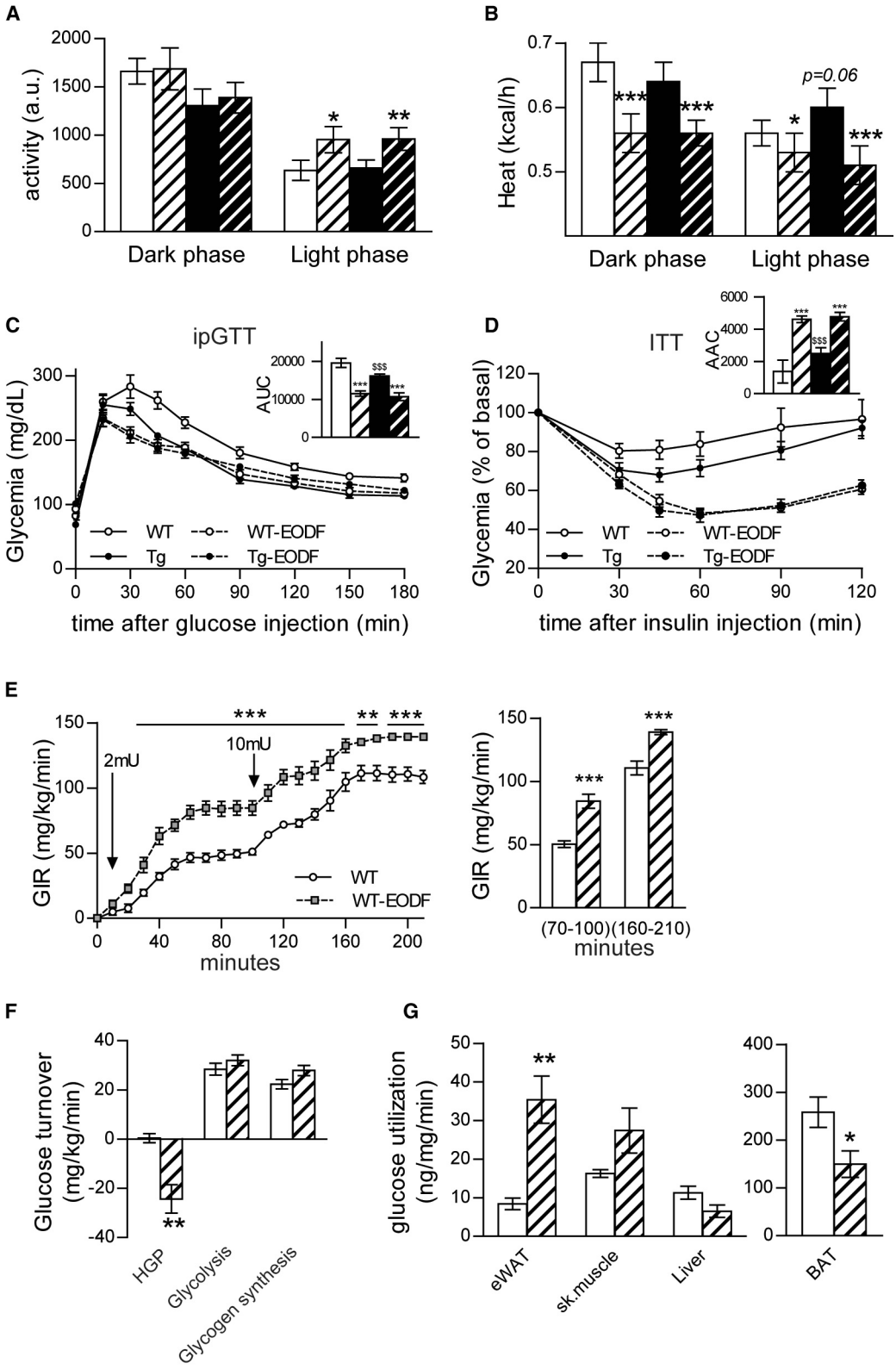
Next, to assess SIRT1 activity, we tested the acetylation status of two well-established SIRT1 targets, RelA/p65 necrosis factor κB (NFκB) and the peroxisome-proliferator-activated receptor (PPAR) γ coactivator 1α (PGC-1α), in different tissues. In general, Tg tissues displayed a lower acetylation state of these substrates, in line with higher SIRT1 activity (Figures S2F and S2G). These effects, however, were more robust in liver than in adipose tissues, while skeletal muscle displayed the more modest effects (Figures S2F and S2G). This might be explained by the differences in SIRT1 protein levels between tissues (Figure S2H), as the absolute increase in SIRT1 protein levels induced by transgenesis was proportional to levels at baseline (Figure S2H). EODF had limited effects on hepatic PGC-1α acetylation levels, but not on RelA/p65 NFκB (Figures S2F and S2G). Importantly, EODF might lead to lower SIRT1 activity in muscle and BAT, as evidenced by the higher acetylation state of PGC-1α and RelA/p65 acetylation under these circumstances (Figures S2F and S2G).

Increased mitochondrial function has been proposed to be a mechanism by which CR promotes health benefits (Nisoli et al., 2005). Hence, we explored how EODF and SIRT1 transgenesis affected mitochondrial function through respirometry analyses in liver, BAT, eWAT, and skeletal muscle. In our protocol, we evaluated Complex I (CI) respiration, Complex I + Complex II (CI + CII) respiration, and maximal electron transport system (ETS) capacity. In skeletal muscle, neither EODF nor SIRT1 transgenesis affected respiratory function (Figure 3B). We also confirmed previous observations indicating a higher CII activity in the livers from Tg mice (Boutant et al., 2015) (Figure 3B). Surprisingly, EODF blunted the higher CII activity in Tg livers (Figure 3B). This was not associated with alterations in CII protein levels (Figure 3A), suggesting possible roles for post-translational modifications in the regulation of liver CII activity in Tg mice. In the BAT, as reported (Boutant et al., 2015), SIRT1 transgenesis increased respiratory capacity in mice fed AL (Figure 3B). However, EODF abolished the effect of SIRT1 overexpression on BAT respiration (Figure 3B), both in the coupled state and in the uncoupled state (data not shown). The decrease in BAT oxidative capacity in EODF mice is in line with their higher lipid accumulation (Figure 1E). Finally, in eWAT, EODF enhanced respiration rates, in association with an increase in CI protein levels both in WT and Tg mice (Figures 3A and 3B). These results illustrate how EODF enhances mitochondrial respiration in eWAT, but not in liver, BAT, or skeletal muscle. Furthermore, SIRT1 overexpression neither mimics nor boosts the effect of EODF on mitochondrial respiration. In BAT, EODF actually opposes the effects of SIRT1 transgenesis.

Comparing SIRT1$^{Tg/Tg}$ Gene Expression Profiles to Those of CR and Exercise Training

SIRT1 activation has been proposed to act as a CR-mimetic strategy. If this was true, the transcriptomic landscapes in Tg mice should display considerable similarities to those of WT mice upon CR or EODF. Hence, we used microarray technologies and hierarchical clustering to interrogate how the gene expression patterns in liver, eWAT, BAT, or skeletal muscle of Tg mice resembled those of WT mice fed AL or under EODF (WT-EODF). We also included an additional group in which WT mice underwent exercise training (EX) for 12 weeks (Figure S3A), to account for another lifestyle intervention, unrelated to CR, that reduces BW and improves glucose homeostasis (Mercken et al., 2012). The efficacy of our training intervention was clear, as trained mice displayed reduced BW due to reduced fat mass (Figures S3B and S3C) and a marked improvement in treadmill performance, maximal oxygen consumption (VO_2 max), glucose tolerance, and insulin sensitivity (Figures S3D and S3H).

In the liver, hierarchical clustering analyses revealed that the gene expression patterns of EODF mice were clearly set apart from those of other groups (Figure 4A). This indicates that EODF has a profound impact on liver gene expression that is



(legend on next page)

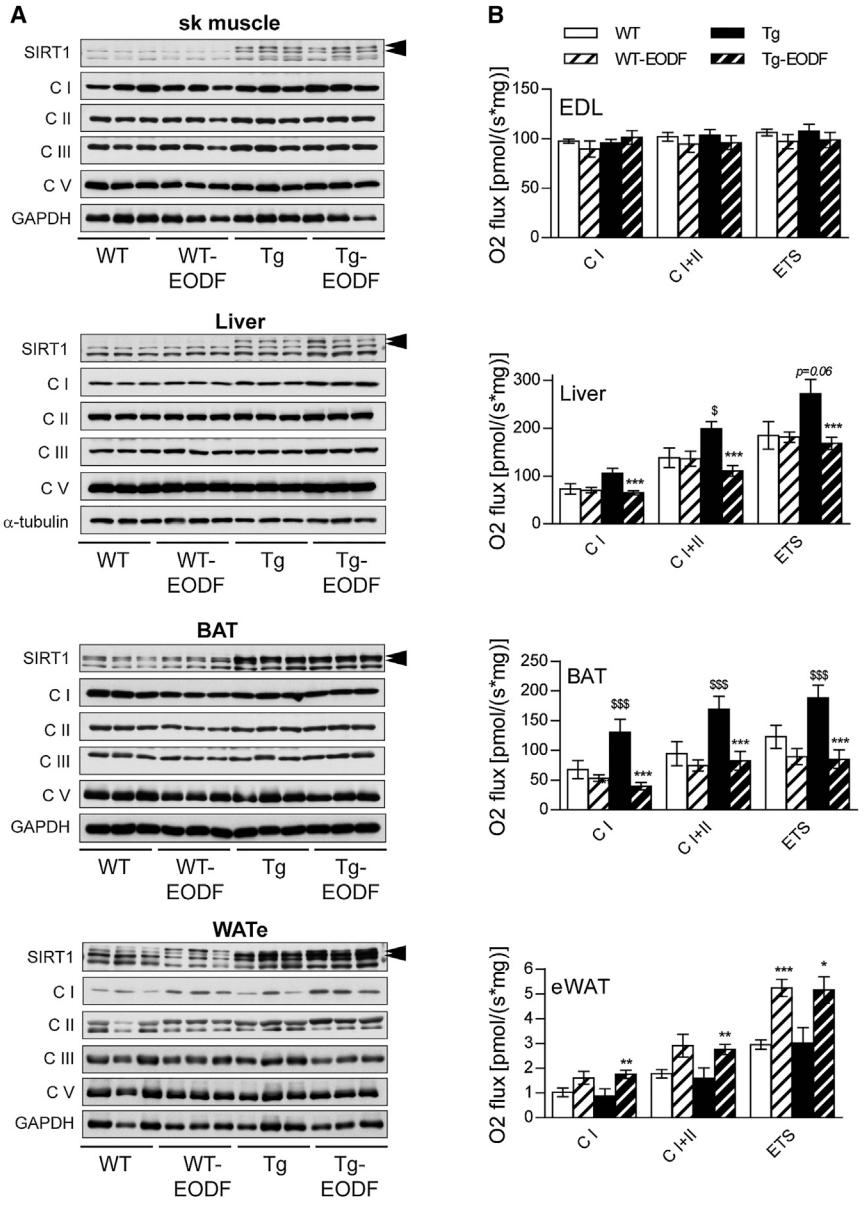


Figure 3. SIRT1 Transgenesis Does Not Enhance EODF Effects on Mitochondrial Metabolism

Mice were euthanized after an overnight fast, and tissues were collected for analysis.

(A) Protein extracts from frozen tissues were used to measure the markers indicated. sk, skeletal.

(B) Respirometry analyses in permeabilized EDL muscle fibers, liver homogenates, BAT homogenates, and eWAT tissue were conducted using high-resolution respirometry. CI respiration, CI + CII respiration, and maximal ETS capacity were examined as described in the Supplemental Experimental Procedures. O_2 flux values are expressed relative to tissue wet weight. All values are presented as mean ± SEM of n = 5–6 mice per genotype and group. *p < 0.05, significant difference between AL- and EODF-fed mice. $ indicates significant difference between genotypes at p < 0.05.

See also Supplemental Experimental Procedures and Figure S2.

not mimicked by SIRT1 transgenesis. In particular, livers from WT-EODF mice displayed marked enrichment in gene sets relating to DNA replication and mismatch repair (Figure S4A; data not shown). In the skeletal muscle, we did not observe major differences between WT, WT-EODF, and Tg mice (Figure 4B). In contrast, EX led to a clearly differentiated gene expression pattern (Figure 4B). Accordingly, and in contrast to SIRT1 transgenesis and EODF, EX enhanced oxidative-phosphorylation (OXPHOS)-related gene expression (Figure S4B) and mitochondrial respiration in skeletal muscle (Figure S3G).

In BAT, EX and EODF led to disparate gene expression profiles. Interestingly, the profile in Tg BAT resembled, to a certain extent, that of exercised mice (Figure 4C), in line with the fact that both interventions enhance mitochondrial respiration in BAT (Figure 3B; Figure S3H). The transcriptomic changes induced by SIRT1 trangenesis and EODF in BAT are diametrically different. Exemplifying this, PPARα target genes, while upregulated in the BAT of Tg mice (Boutant et al., 2015), were downregulated by EODF (Figure S4C). As in liver, EODF promoted dramatic changes in

Figure 2. SIRT1 Overexpression Does Not Mimic the Metabolic Changes of EODF

(A and B) Daily activity (A) and heat production (B) of mice were recorded using a comprehensive laboratory animal monitoring system (CLAMS).

(C and D) Intraperitoneal glucose tolerance test (ipGTT) (C) and intraperitoneal insulin tolerance test (ITT) (D) analyses were performed as described in the Supplemental Experimental Procedures. Analyses of area under the curve (AUC) and area above the curve (AAC) are present on the top right, respectively.

(E–G) Hyper-insulinemic-euglycemic clamps were performed on WT and WT-EODF mice. Glucose infusion rate (GIR) measured at two different levels of insulin infusion (2 and 10 mU/kg/min) (E), glucose fluxes (F), and glucose uptake in different tissues—epididymal WAT, skeletal (sk) muscle, liver, and BAT—(G) are represented.

All values are presented as mean ± SEM of n = 6–12 mice for each genotype and group. $^{$}p < 0.05$, significant difference between genotypes. *p < 0.05, statistical significant difference between AL- and EODF-fed mice; **p < 0.03, statistical significant difference between AL- and EODF-fed mice; ***p < 0.01, statistical significant difference between AL- and EODF-fed mice. $^{$$$}p<0.01$, statistical significant difference between genotypes.

See also Supplemental Experimental Procedures and Figure S1.

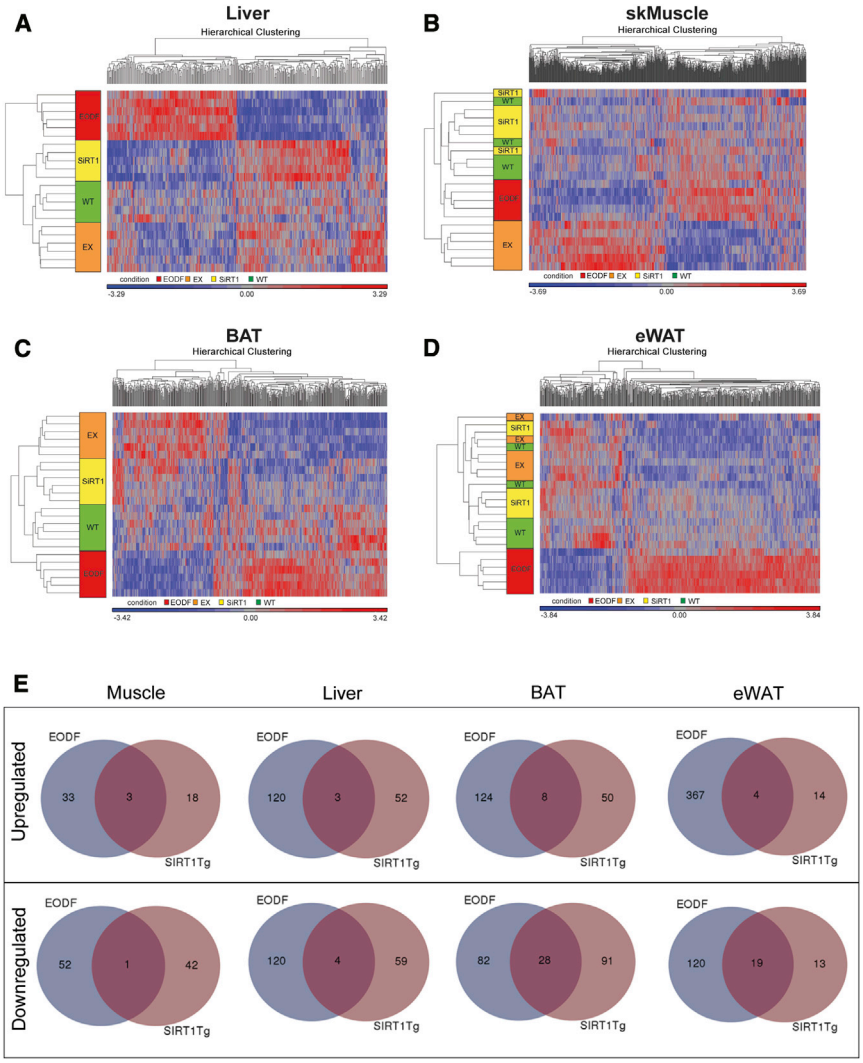


Figure 4. Transcriptomic Analyses Comparing the Effects of CR, EX, and SIRT1 Transgenesis

(A–D) Microarrays were performed on liver (A), skeletal muscle (B), BAT (C), and eWAT (D) from WT, Tg, WT-EODF, and WT-EX mice. Hierarchical clustering was performed based on the most significant transcripts selected by the pairwise comparisons.

(E) Venn diagrams representing the overlap between upregulated or downregulated genes in response to EODF and SIRT1 transgenesis versus WT mice.

See also Figures S3 and S4 and Tables S1 and S2.

eWAT transcription profiles that clearly were segregated from those of the other groups (Figure 4D). In line with the respirometry tests, OXPHOS gene sets were very significantly upregulated in the eWAT upon EODF (Figure S4D). Altogether, these data suggest that (1) EODF promotes dramatic transcriptomic changes in liver, WAT, and BAT, but not in skeletal muscle; (2) the gene expression profile changes induced by SIRT1 transgenesis do not mimic those of EODF; and (3) SIRT1 transgenesis does not mimic the transcriptional effects of EX in skeletal muscle, although similarities were observed in BAT.

Next, we evaluated the overlap between the gene expression changes elicited by EODF and SIRT1 transgenesis compared to WT mice. There was a very marginal overlap between the genes that are significantly upregulated or downregulated in response to EODF and SIRT1 transgenesis (Figure 4E; Figures S4E–S4H; Tables S1 and S2). In addition, while a number of genes were significantly affected by both EODF and SIRT1 transgenesis, many of them were regulated in opposite directions (Tables S1 and S2). This is especially the case in liver, where out of the 51 genes regulated by both EODF and SIRT1 transgenesis, 44 did so in opposing ways (Table S1). Interestingly, SIRT1 was significantly downregulated at the mRNA level in BAT upon EODF (Table S2). Also, as predicted by hierarchical clustering, liver, BAT, and eWAT are the tissues where EODF elicited a higher number of significant transcriptional changes (Figure 4E). In contrast, SIRT1 transgenesis had minor effects on eWAT, while BAT displayed the highest number of significant changes. Hence, the transcriptional changes induced by EODF are largely different from, and even contradictory to, those induced by SIRT1 transgenesis.

DISCUSSION

SIRT1 loss-of-function models suggest that CR requires SIRT1 to promote a number of metabolic and behavioral adaptations (Boily et al., 2008; Chen et al., 2005; Mercken et al., 2014). This led to the speculation that SIRT1-activating strategies could act as CR mimetic. SIRT1 activation via STACs has further strengthened this notion, as they improve insulin sensitivity (Mercken et al., 2012), a paradigm of CR. However, overlapping effects, such as increased insulin sensitivity, do not necessarily indicate mimetic strategies, as what happens between EX and EODF (Figure 4).

Here, we demonstrate that genetic SIRT1 gain of function neither mimics nor boosts the metabolic effects of EODF. EODF and SIRT1 transgenesis lead to opposite effects in BAT, as revealed by histology, metabolic, transcriptomic, and respirometry analyses. In turn, EODF leads to dramatic changes in the transcriptional profiles of liver and eWAT, which are barely affected in Tg mice, at least at the ages tested in our study. Also, both EODF and canonical CR (Chen et al., 2005) lead to increased daily activity, while SIRT1 transgenesis decreases daily activity (Banks et al., 2008; Boutant et al., 2015). These observations argue that SIRT1 is required for the adaptations to CR, but the activation of SIRT1 is not, per se, a CR mimetic. In line with this, SIRT1 transgenesis did not lead to increased lifespan, a well-recognized effect of CR regimes (Herranz et al.,

2010). Additionally, SIRT1 transgenesis also failed to mimic or enhance the protective effects of EODF on cancer in p53 heterozygous mice (Herranz et al., 2011).

Our results indicate that endogenous SIRT1 levels are enough to fully adapt to EODF. This is in line with previous observations in other SIRT1 transgenic mouse models. By submitting muscle-specific SIRT1 KO mice to a 40% decrease in daily caloric intake, it was shown that CR requires SIRT1 to enhance insulin-stimulated muscle glucose uptake (Schenk et al., 2011). Under similar regimes, however, muscle-specific SIRT1 overexpression did not improve CR-induced adaptations (White et al., 2013). In agreement, CR failed to enhance lifespan in SIRT1 KO mice, but heterozygous expression of SIRT1 was enough to ensure similar CR-induced lifespan extension as in WT mice (Mercken et al., 2014).

While EODF and classic CR have been commonly used as CR regimes, a molecular underpinning of potential differences between these interventions has never been fully addressed. In this sense, several studies have reported changes in SIRT1 expression in liver, WAT, and muscle upon CR (Chen et al., 2008; Nisoli et al., 2005) that were not observed in our EODF study. Nevertheless, it has been shown that EODF and CR display a very significant overlap in their transcriptional profiles for different tissues, including liver, muscle, and WAT (Pearson et al., 2008). Similarly, classic CR and EODF render very comparable outcomes on glucose and lipid management (Mattson and Wan, 2005), even in humans (Barnosky et al., 2014). Despite these observations, further efforts will be needed to evaluate whether the discrepancies between the SIRT1 gain of function and EODF scenarios can be extrapolated to other CR regimes.

The impact of CR on mitochondrial biogenesis has also been a matter of debate (Hancock et al., 2011). In our model, EODF triggered modest increases in mitochondrial function in eWAT, but not in skeletal muscle, liver, or BAT. SIRT1 has been shown to regulate mitochondrial biogenesis in skeletal muscle cells via the deacetylation of PGC-1α (Cantó et al., 2009; Gerhart-Hines et al., 2007). However, muscle-specific SIRT1 KO mice do not display major differences in mitochondrial marker levels (Menzies et al., 2013; Philp et al., 2011). Supra-physiological overexpression of SIRT1 in skeletal muscle has rendered conflicting results, as both increases (Chalkiadaki et al., 2014; Price et al., 2012) and decreases (Gurd et al., 2009) in muscle oxidative capacity have been reported. This might be due to the different strategies used to overexpress SIRT1 in these studies. However, in line with previous reports (Boutant et al., 2015; Brandon et al., 2015; Pfluger et al., 2008; White et al., 2014), our results suggest that mild SIRT1 overexpression does not enhance mitochondrial biogenesis in skeletal muscle.

Altogether, this study demonstrates that, while SIRT1 activation holds a strong therapeutic promise for the management of metabolic disease, the source of these benefits can be clearly disentangled from those of CR regimes in the shape of intermittent fasting.

EXPERIMENTAL PROCEDURES

Animal Care

SIRT1 Tg mice expressing a single additional copy of the SIRT1 gene per allele ($SIRT1^{Tg/Tg}$) have already been described (Boutant et al., 2015). Only male mice were used. Mice were kept in a standard temperature- and humidity-controlled environment with a 12-hr:12-hr light:dark cycle. Mice had nesting material and AL access to water and a commercial low-fat diet (LFD) (D12450J, Research Diets). The CR study was performed on 12-week-old mice as EODF. At the beginning of the experiment, food was removed from the hopper and returned approximately 24 hr later. This pattern of food removal and return took place daily for 3 months. The phenotyping started after 12 weeks of EODF, and mice were sacrificed after a total of 18 weeks of CR and an overnight fast. Overnight fasts prior to tests and sacrifice were always done after the feeding day in the EODF group. All animal experiments were carried out according to national Swiss and EU ethical guidelines and approved by the local animal experimentation committee under license number 2519. Phenotyping procedures can be found in the Supplemental Experimental Procedures.

Transcriptomic Analyses

Illumina microarrays were used to profile the gene expression levels of approximately 25,000 genes in the liver, skeletal muscle, BAT, and eWAT of WT and $SIRT1^{Tg/Tg}$ mice (n = 6 per group). The RNA samples were prepared using the Illumina TotalPrep RNA Amplification Kit and were then hybridized on MouseRef-8 v2.0 Expression BeadChips. Raw microarray data were analyzed for quality control and extracted using the Illumina GenomeStudio Software, and they were then analyzed using Partek Genomics Suite (GS) software. Basically, raw data were $\log_2$ transformed and quantile normalized prior to statistical analysis. Statistics consisted of a one-way ANOVA in each tissue sample followed by pairwise comparisons. The Benjamini-Hochsberg multiple testing correction method was applied. A corrected p value <0.05 was considered as significant. Venn diagrams were generated using a free online tool (http://bioinformatics.psb.ugent.be/webtools/Venn/).

Statistical Analyses

Statistical analyses were performed with Prism software (GraphPad). Differences between two groups were analyzed using Student's t test (two-tailed), and multiple comparisons were analyzed by ANOVA with a Bonferroni post hoc test. A p value <0.05 was considered significant. Data are expressed as means ± SEM.

Further experimental procedures can be found in the Supplemental Information.

ACCESSION NUMBERS

The accession number for the results of the microarray reported in this paper is GEO: GSE70857.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.02.007.

AUTHOR CONTRIBUTIONS

M.B., S.S.K., and C.C. performed animal experimentation and tissue collection. M.B., M.J., and C.C. performed molecular biology and respirometry analyses. F.R., S.M., and P.D. performed microarray analyses. M.B. and C.C. experimentally conceived of the project and wrote the manuscript. All authors contributed to editing duties.

ACKNOWLEDGMENTS

We thank the members of the EPFL animal house for technical support. We would also like to thank Prof. Manuel Serrano (CNIO, Madrid), who provided the $SIRT1^{Tg/Tg}$ mice and valuable comments on this work. We also thank Physiogenex for their help with hyperinsulinemic-euglycemic clamps. All authors are employees of the Nestlé Institute of Health Sciences.

Received: July 14, 2015
Revised: November 3, 2015
Accepted: January 26, 2016
Published: February 25, 2016

REFERENCES

Banks, A.S., Kon, N., Knight, C., Matsumoto, M., Gutiérrez-Juárez, R., Rossetti, L., Gu, W., and Accili, D. (2008). SirT1 gain of function increases energy efficiency and prevents diabetes in mice. Cell Metab. *8*, 333–341.

Barnosky, A.R., Hoddy, K.K., Unterman, T.G., and Varady, K.A. (2014). Intermittent fasting vs daily calorie restriction for type 2 diabetes prevention: a review of human findings. Transl. Res. *164*, 302–311.

Bartfai, T., and Conti, B. (2012). Molecules affecting hypothalamic control of core body temperature in response to calorie intake. Front. Genet. *3*, 184.

Boily, G., Seifert, E.L., Bevilacqua, L., He, X.H., Sabourin, G., Estey, C., Moffat, C., Crawford, S., Saliba, S., Jardine, K., et al. (2008). SirT1 regulates energy metabolism and response to caloric restriction in mice. PloS One *3*, e1759.

Boutant, M., Joffraud, M., Kulkarni, S.S., García-Casarrubios, E., García-Roves, P.M., Ratajczak, J., Fernández-Marcos, P.J., Valverde, A.M., Serrano, M., and Cantó, C. (2015). SIRT1 enhances glucose tolerance by potentiating brown adipose tissue function. Mol. Metab. *4*, 118–131.

Brandon, A.E., Tid-Ang, J., Wright, L.E., Stuart, E., Suryana, E., Bentley, N., Turner, N., Cooney, G.J., Ruderman, N.B., and Kraegen, E.W. (2015). Overexpression of SIRT1 in rat skeletal muscle does not alter glucose induced insulin resistance. PLoS ONE *10*, e0121959.

Cantó, C., Gerhart-Hines, Z., Feige, J.N., Lagouge, M., Noriega, L., Milne, J.C., Elliott, P.J., Puigserver, P., and Auwerx, J. (2009). AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature *458*, 1056–1060.

Chalkiadaki, A., Igarashi, M., Nasamu, A.S., Knezevic, J., and Guarente, L. (2014). Muscle-specific SIRT1 gain-of-function increases slow-twitch fibers and ameliorates pathophysiology in a mouse model of duchenne muscular dystrophy. PLoS Genet. *10*, e1004490.

Chen, D., Steele, A.D., Lindquist, S., and Guarente, L. (2005). Increase in activity during calorie restriction requires Sirt1. Science *310*, 1641.

Chen, D., Bruno, J., Easlon, E., Lin, S.J., Cheng, H.L., Alt, F.W., and Guarente, L. (2008). Tissue-specific regulation of SIRT1 by calorie restriction. Genes Dev. *22*, 1753–1757.

Cohen, H.Y., Miller, C., Bitterman, K.J., Wall, N.R., Hekking, B., Kessler, B., Howitz, K.T., Gorospe, M., de Cabo, R., and Sinclair, D.A. (2004). Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. Science *305*, 390–392.

Gerhart-Hines, Z., Rodgers, J.T., Bare, O., Lerin, C., Kim, S.H., Mostoslavsky, R., Alt, F.W., Wu, Z., and Puigserver, P. (2007). Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. EMBO J. *26*, 1913–1923.

Gurd, B.J., Yoshida, Y., Lally, J., Holloway, G.P., and Bonen, A. (2009). The deacetylase enzyme SIRT1 is not associated with oxidative capacity in rat heart and skeletal muscle and its overexpression reduces mitochondrial biogenesis. J. Physiol. *587*, 1817–1828.

Hancock, C.R., Han, D.H., Higashida, K., Kim, S.H., and Holloszy, J.O. (2011). Does calorie restriction induce mitochondrial biogenesis? A reevaluation. FASEB J. *25*, 785–791.

Herranz, D., Muñoz-Martin, M., Cañamero, M., Mulero, F., Martinez-Pastor, B., Fernandez-Capetillo, O., and Serrano, M. (2010). Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. Nat. Commun. *1*, 3.

Herranz, D., Iglesias, G., Muñoz-Martín, M., and Serrano, M. (2011). Limited role of Sirt1 in cancer protection by dietary restriction. Cell Cycle *10*, 2215–2217.

Mattson, M.P., and Wan, R. (2005). Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. J. Nutr. Biochem. *16*, 129–137.

McBurney, M.W., Yang, X., Jardine, K., Hixon, M., Boekelheide, K., Webb, J.R., Lansdorp, P.M., and Lemieux, M. (2003). The mammalian SIR2alpha protein has a role in embryogenesis and gametogenesis. Mol. Cell. Biol. *23*, 38–54.

Menzies, K.J., Singh, K., Saleem, A., and Hood, D.A. (2013). Sirtuin 1-mediated effects of exercise and resveratrol on mitochondrial biogenesis. J. Biol Chem. *288*, 6968–6979.

Mercken, E.M., Carboneau, B.A., Krzysik-Walker, S.M., and de Cabo, R. (2012). Of mice and men: the benefits of caloric restriction, exercise, and mimetics. Ageing Res. Rev. *11*, 390–398.

Mercken, E.M., Hu, J., Krzysik-Walker, S., Wei, M., Li, Y., McBurney, M.W., de Cabo, R., and Longo, V.D. (2014). SIRT1 but not its increased expression is essential for lifespan extension in caloric-restricted mice. Aging Cell *13*, 193–196.

Nisoli, E., Tonello, C., Cardile, A., Cozzi, V., Bracale, R., Tedesco, L., Falcone, S., Valerio, A., Cantoni, O., Clementi, E., et al. (2005). Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. Science *310*, 314–317.

Pacholec, M., Bleasdale, J.E., Chrunyk, B., Cunningham, D., Flynn, D., Garofalo, R.S., Griffith, D., Griffor, M., Loulakis, P., Pabst, B., et al. (2010). SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. J. Biol. Chem. *285*, 8340–8351.

Pearson, K.J., Baur, J.A., Lewis, K.N., Peshkin, L., Price, N.L., Labinskyy, N., Swindell, W.R., Kamara, D., Minor, R.K., Perez, E., et al. (2008). Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab. *8*, 157–168.

Pfluger, P.T., Herranz, D., Velasco-Miguel, S., Serrano, M., and Tschöp, M.H. (2008). Sirt1 protects against high-fat diet-induced metabolic damage. Proc. Natl. Acad. Sci. USA *105*, 9793–9798.

Philp, A., Chen, A., Lan, D., Meyer, G.A., Murphy, A.N., Knapp, A.E., Olfert, I.M., McCurdy, C.E., Marcotte, G.R., Hogan, M.C., et al. (2011). Sirtuin 1 (SIRT1) deacetylase activity is not required for mitochondrial biogenesis or peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha) deacetylation following endurance exercise. J. Biol. Chem. *286*, 30561–30570.

Price, N.L., Gomes, A.P., Ling, A.J.Y., Duarte, F.V., Martin-Montalvo, A., North, B.J., Agarwal, B., Ye, L., Ramadori, G., Teodoro, J.S., et al. (2012). SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. Cell Metab. *15*, 675–690.

Schenk, S., McCurdy, C.E., Philp, A., Chen, M.Z., Holliday, M.J., Bandyopadhyay, G.K., Osborn, O., Baar, K., and Olefsky, J.M. (2011). Sirt1 enhances skeletal muscle insulin sensitivity in mice during caloric restriction. J. Clin. Invest. *121*, 4281–4288.

White, A.T., McCurdy, C.E., Philp, A., Hamilton, D.L., Johnson, C.D., and Schenk, S. (2013). Skeletal muscle-specific overexpression of SIRT1 does not enhance whole-body energy expenditure or insulin sensitivity in young mice. Diabetologia *56*, 1629–1637.

White, A.T., Philp, A., Fridolfsson, H.N., Schilling, J.M., Murphy, A.N., Hamilton, D.L., McCurdy, C.E., Patel, H.H., and Schenk, S. (2014). High-fat diet-induced impairment of skeletal muscle insulin sensitivity is not prevented by SIRT1 overexpression. Am. J. Physiol. Endocrinol. Metab. *307*, E764–E772.